

WHAT IS CLAIMED IS:

1. An isolated or recombinant polypeptide comprising an amino acid sequence of an extracellular domain, wherein said extracellular domain amino acid sequence has at least about 75% amino acid sequence identity to an extracellular domain amino acid sequence of at least one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, and is not a naturally-occurring extracellular domain amino acid sequence, and wherein said polypeptide has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.
- 10 2. The isolated or recombinant polypeptide of claim 1, wherein said extracellular domain (ECD) amino acid sequence has at least about 90% sequence identity to an ECD amino acid sequence of at least one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.
- 15 3. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an ECD amino acid sequence of any one of SEQ ID NOS:48-68, 174-182, 184-221, 283-285, and 290-293.
- 20 4. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an amino acid sequence of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.
- 25 5. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.
- 30 6. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.
7. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has a decreased or a lowered binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

8. The isolated or recombinant polypeptide of claim 1, 5, 6, or 7, wherein the polypeptide has an ability to induce T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation and further comprises at least transmembrane domain (TMD) and/or a cytoplasmic, wherein said transmembrane domain is not a naturally-occurring TMD.

9. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide induces T-cell proliferation.

10 10. The isolated or recombinant polypeptide of claim 1, 5, 6, or 7, wherein the polypeptide has an ability to induce a T-cell proliferative response about equal to or greater than that of human B7-1.

15 11. The isolated or recombinant polypeptide of claim 1, wherein the polypeptide has an ability to modulate T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

20 12. The isolated or recombinant polypeptide of claim 5, which polypeptide comprises an extracellular domain amino acid sequence of any one of SEQ ID NOS:48-68 and 174-209.

25 13. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an extracellular domain (ECD) amino acid sequence encoded by an ECD coding nucleotide sequence, the ECD coding nucleotide sequence selected from the group of:

(a) a nucleotide sequence comprising a nucleotide fragment of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142, wherein said nucleotide fragment encodes an ECD amino acid sequence;

30 (b) a nucleotide sequence that encodes the ECD amino acid sequence of a polypeptide selected from any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and

(c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

- 5 14. An isolated or recombinant polypeptide, which polypeptide comprises a non-naturally-occurring amino acid sequence encoded by a nucleic acid comprising a polynucleotide sequence selected from the group of:
- (a) a polynucleotide sequence selected from SEQ ID NOS:1-21 and 95-142, or a complementary polynucleotide sequence thereof;
- 10 (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary polynucleotide sequence thereof;
- 15 (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent or highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b);
- (d) a polynucleotide sequence comprising all or a nucleotide fragment of (a), (b), or (c), wherein the nucleotide fragment encodes a polypeptide having a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1;
- 20 (e) a polynucleotide sequence encoding a polypeptide, the polypeptide comprising an amino acid sequence which is substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and
- (f) a polynucleotide sequence encoding a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1, which polynucleotide sequence has at least about 70% identity to at least one polynucleotide sequence of (a), (b), (c), or (d).

- 30 15. The isolated or recombinant polypeptide of claim 14, the polypeptide comprising an amino acid sequence of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

- 10026244-1222004
16. The isolated or recombinant polypeptide of claim 14, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.
- 5 17. The isolated or recombinant polypeptide of claim 14 or 16, wherein the polypeptide induces T-cell proliferation.
- 10 18. The isolated or recombinant polypeptide of claim 14 or 16, wherein the polypeptide induces a T-cell proliferative response about equal to or greater than that of human B7-1.
- 15 19. An isolated or recombinant polypeptide comprising a amino acid sequence according to the formula:
- MGHTM-X6-W-X8-SLPPK-X14-PCL-X18-X19-X20-QLLVLT-X27-
LFYFCSGITPKSVTKRVKETVMLSCDY-X55-TSTE-X60-LTSLRIYW-X69-
KDSKMLVAILPGKVQVWPEYKNRTITDMNDN-X101-RIVI-X106-ALR-X110-SD-
X113-GTYTCV-X120-QKP-X124-LKGAYKLEHL-X135-SVRLMIRADFPVP-X149-
X150-X151-DLGNPSPNIRRLICS-X167-X168-X169-GFPRPHL-X177-
WLENGEELNATNTT-X192-SQDP-X197-T-X199-LYMSSEL-X208-FNVTNN-X215-
20 SI-X218-CLIKYGEL-X227-VSQIFPWSPKPKQEPPIDQLPF-X249-VIIPVSGALVL-X261-
A-X263-VLY-X267-X268-ACRH-X273-ARWKRTTRNEETVGTE RLSPIYLGSAQSSG
(SEQ ID NO:284), or a subsequence thereof comprising an extracellular domain, wherein
position X6 is Lys or Glu; position X8 is Arg or Gly; position X14 is Arg or Cys; position X18 is Trp or Arg; position X19 is Pro or Leu; position X20 is Ser or Pro;
position X27 is Asp or Gly; position X55 is Asn or Ser; position X60 is Glu or Lys; position X69 is Gln or Arg; position X101 is Pro or Leu; position X106 is Leu or Gln; position X110 is Pro or Leu; position X113 is Lys or Ser; position X120 is Val or Ile; position X124 is Val or Asp; position X135 is Thr or Ala; position X149 is Thr, Ser, or del; position X150 is Ile or del; position X151 is Asn or Thr; position X167 is Thr or del; position X169 is Ser or del; position X169 is Gly or del; position X177 is Cys or Tyr; position X192 is Val or Leu; position X197 is Gly or Glu; position X199 is Glu or Lys; position X208 is Gly or Asp; position X215 is His or Arg; position X218 is Ala or Val; position X227 is Ser or Leu; position X249 is Trp, Leu, or Arg; position X261 is Ala or Thr; position X263 is Val, Ala,

or Ile; position X267 is Arg or Cys; position X268 is Pro or Leu; and position X273 is Gly or Val.

20. The isolated or recombinant polypeptide of claim 19, which
5 polypeptide comprises an extracellular domain amino acid sequence of any one of SEQ ID NOS:51-56, 58, 61, 66, 67, 174-179, 181, 185-187, 189, 192-194, 197, 199, 202, 205, 208, 215, 217, 220, and 285.

21. The isolated or recombinant polypeptide of claim 19, wherein the
10 polypeptide has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

22. The isolated or recombinant polypeptide of claim 19 or 21, wherein
the polypeptide induces T-cell proliferation.
15

23. The isolated or recombinant polypeptide of claim 19 or 21, wherein
the polypeptide induces a T-cell proliferative response about equal to or greater than that of
human B7-1.

20 24. The isolated or recombinant polypeptide of claim 19, comprising
three or more of: Lys at position X6; Arg at position X8; Arg at position X14; Trp at
position X18; Pro at position X19; Ser at position X20; Asp at position X27; Asn at
position X55; Leu at position X106; Pro at position X110; Lys at position X113; Val at
position X120; Val at position X124; Thr at position X135; Asn at position X151; Cys at
25 position X177; Val at position X192; Gly at position X197; Glu at position X199; Gly at
position X208; His at position X215; Ala at position X218; Trp at position X249; Ala at
position X261; Val at position X263; Arg at position X267; Pro at position X268; and
Gly at position X273.

30 25. The isolated or recombinant polypeptide of claim 24, comprising
three or more of: Arg at position X8; Arg at position X14; Trp at position X18; Pro at
position X19; Ser at position X20; Pro at position X110; Val at position X120; Val at
position X124; Cys at position X177; Val at position X192; Gly at position X197; Glu at

position X199; Gly at position X208; His at position X215; Ala at position X218; Trp at position X249; Ala at position X261; and Val at position X263.

26. The isolated or recombinant polypeptide of claim 25, comprising the
5 extracellular domain amino acid sequence of SEQ ID NO:66 or SEQ ID NO:285.

27. The isolated or recombinant polypeptide of claim 25, comprising the entire amino acid sequence of SEQ ID NO:66 or SEQ ID NO:285.

10 28. An isolated or recombinant polypeptide comprising a subsequence of an amino acid sequence set forth in any of SEQ ID NOS:48-68, 174-182, 184-221, 283-285, and 290-293, wherein the subsequence is the extracellular domain of said amino acid sequence.

15 29. The isolated or recombinant polypeptide of claim 1, 14, 19, or 28, further comprising a signal peptide sequence.

20 30. The polypeptide of claim 29, wherein the signal peptide sequence is selected from the signal peptide sequence set forth in any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

25 31. The polypeptide of claim 30, further comprising a transmembrane domain amino acid sequence or a cytoplasmic domain amino acid sequence selected from the transmembrane domain amino acid sequence or the cytoplasmic domain amino acid sequence set forth in any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

32. The polypeptide of claim 1, 14, 19, or 28 comprising a soluble extracellular domain, wherein said polypeptide or ECD thereof binds CD28 and/or CTLA-4.

30 33. The polypeptide of claim 1, 14, 19, 28, 29, or 31, wherein the polypeptide comprises a fusion protein comprising at least one additional amino acid sequence.

34. The polypeptide of claim 33, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.

5 35. The polypeptide of claim 34, wherein the at least one Ig polypeptide comprises at least one human IgG polypeptide comprising an Fc hinge, a CH₂ domain, and a CH₃ domain.

10 36. The polypeptide of claim 1, 14, 19, 28, 29, or 31, comprising a polypeptide purification subsequence.

15 37. The polypeptide of claim 36, wherein the polypeptide purification subsequence is selected from: an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion.

20 38. The polypeptide of claim 1, 14, 19, 28, 29, or 31 comprising a modified amino acid.

25 39. The polypeptide of claim 38, wherein the modified amino acid is selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

40. A composition comprising at least one polypeptide of claim 38 and a pharmaceutically acceptable excipient.

25 41. A composition comprising at least one polypeptide of claim 1, 14, 19, 28, 29, or 31 and a pharmaceutically acceptable excipient.

30 42. A composition comprising:

an isolated or recombinant polypeptide comprising an amino acid sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, 290-293, or a costimulatory fragment thereof, wherein said polypeptide or costimulatory fragment has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4

binding affinity ratio of human B7-1 or an ability to induce a T cell proliferation response equal to or greater than that induced by human B7-1, and
a carrier.

- 5 43. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from:
- (a) a polynucleotide sequence selected from SEQ ID NOS:1-21 and 95-142, or a complementary polynucleotide sequence thereof;
- (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID 10 NOS:48-68, 174-221, 283-285, and 290-293, or a complementary polynucleotide sequence thereof;
- (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); and
- 15 (d) a polynucleotide sequence comprising all or a nucleotide fragment of (a), (b), or (c), wherein the nucleotide fragment encodes a polypeptide having a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1 or a polypeptide having an ability to induce a T cell proliferation response that is about equal to or greater than that induced by human B7-1.

- 20 44. An isolated or recombinant nucleic acid comprising a polynucleotide sequence encoding a polypeptide, wherein the encoded polypeptide comprises an amino acid sequence which is substantially identical over at least about 150 contiguous amino acid residues of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293 and is a non naturally-occurring amino acid sequence.

- 25 45. The nucleic acid of claim 44, wherein the encoded polypeptide is substantially identical over at least about 175 contiguous amino acid residues of any one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.

- 30 46. An isolated or recombinant nucleic acid comprising a nucleotide sequence coding for a polypeptide comprising the amino acid sequence set forth in any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a subsequence thereof, wherein the subsequence comprises at least one of: the signal sequence of said polypeptide, the

1

extracellular domain of said polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of said polypeptide, and wherein the amino acid sequence or subsequence is a non naturally-occurring sequence.

5 47. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1 or an ability to induce a T cell proliferation response equal to or greater than that of human B7-1.

10 48. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

15 49. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide has a decreased or a lowered binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

20 50. The nucleic acid of claim 43, 44, 46, or 49, wherein the encoded polypeptide induces T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

25 51. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

52. The nucleic acid of claim 43, 44, or 46, wherein the nucleic acid encodes a fusion protein comprising at least one additional amino acid sequence.

30 53. The nucleic acid of claim 52, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
54. The nucleic acid of claim 53, wherein the at least one Ig polypeptide comprises at least one human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.
- 5 55. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide comprises a signal sequence.
- 10 56. The nucleic acid of claim 43, 44, 46, or 49, wherein the encoded polypeptide comprises a precursor peptide.
57. The nucleic acid of claim 43, 44, or 46, wherein the encoded polypeptide comprises an epitope tag sequence.
- 15 58. A cell comprising the nucleic acid of claim 43, 44, 46, or 49.
59. The cell of claim 58, wherein the cell expresses a polypeptide encoded by the nucleic acid.
60. A vector comprising the nucleic acid of claim 43, 44, 46, or 49.
- 20 61. The vector of claim 60, wherein the vector comprises a plasmid, a cosmid, a phage, a virus, a virus-like particle, or a fragment of a virus.
62. The vector of claim 60, wherein the vector is an expression vector.
- 25 63. The expression vector of claim 62, wherein the nucleic acid is operably linked to a promoter.
64. The expression vector of claim 62, further comprising a polynucleotide sequence encoding an antigen.
- 30 65. The expression vector of claim 64, wherein the antigen is a cancer antigen.

66. The expression vector of claim 64, wherein the nucleic acid is operably linked to first promoter and the polynucleotide sequence encoding the antigen is operably linked to a second promoter.

5 67. The expression vector of claim 65, wherein the cancer antigen is EpCam/KSA or a mutant or variant thereof.

10 68. The expression vector of claim 67, wherein the expression vector comprises the vector shown in Figure 22B.

69. A host cell comprising the vector of claim 60.

15 70. A composition comprising the nucleic acid of claim 43, 44, 46, or 49 and an excipient.

71. The composition of claim 70, wherein the excipient is a pharmaceutically acceptable excipient.

20 72. A composition of matter comprising at least one nucleic acid of claim 43, 44, 46, or 49.

73. The composition of claim 72, wherein the composition comprises a library comprising at least about 2, 5, 10, 50 or more nucleic acids.

25 74. A composition produced by cleaving at least one nucleic acid of claim 43, 44, or 46.

75. The composition of claim 74, wherein the cleaving comprises mechanical, chemical, or enzymatic cleavage.

30 76. The composition of claim 75, wherein the enzymatic cleavage comprises cleavage with a restriction endonuclease, an RNase, or a DNase.

- 1000000000000000
77. A composition produced by a process comprising incubating at least one nucleic acid of claim 43, 44, or 46 in the presence of deoxyribonucleotide triphosphates and a nucleic acid polymerase.
- 5 78. The composition of claim 77, wherein the nucleic acid polymerase is a thermostable polymerase.
- 10 79. An isolated or recombinant nucleic acid encoding a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1, produced by mutating or recombining at least one nucleic acid of claim 43, 44, or 46.
- 15 80. An isolated or recombinant polypeptide comprising an amino acid sequence having at least about 95% identity to at least about one of SEQ ID NOS:69-92, 222-252, 286-289, or a subsequence thereof comprising an extracellular domain, wherein said amino acid sequence (a) is a non naturally-occurring amino acid sequence, and (b) comprises at least one of:
- Gly at position 2; Thr at position 4; Arg at position 5; Gly at position 8; Pro at position 12; Met at position 25; Cys at position 27; Pro at position 29; Leu at position 31; Arg at position 40; Leu at position 52; His at position 65; Ser at position 78; Asp at position 80; Tyr at position 87; Lys at position 120; Asp at position 122; Lys at position 129; Met at position 135; Phe at position 150; Ile at position 160; Ala at position 164; His at position 172; Phe at position 174; Leu at position 176; Asn at position 178; Asn at position 186; Glu at position 194; Gly at position 196; Thr at position 199; Ala at position 210; His at position 212; Arg at position 219; Pro at position 234; Asn at position 241; Leu at position 244; Thr at position 250; Ala at position 254; Tyr at position 265; Arg at position 266; Glu at position 273; Lys at position 275; Ser at position 276; an amino acid deletion at position 276; or Thr at position 279, wherein the position number corresponds to that of the human B7-1 amino acid sequence (SEQ ID NO:278),
- 20 30 wherein said polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1 or an ability to induce a T cell proliferation response that is equal to or less than that of human B7-1.

1
0
0
3
2
2
1
-
1
2
2
0
0
1

81. The isolated or recombinant polypeptide of claim 80, wherein said polypeptide comprises an amino acid sequence having at least about 98% identity to at least one of SEQ ID NOS:69-92, 222-252, 286-289, or a subsequence thereof comprising an extracellular domain, said amino acid sequence comprising at least one of:

Gly at position 2; Thr at position 4; Arg at position 5; Gly at position 8; Pro at position 12; Met at position 25; Cys at position 27; Pro at position 29; Leu at position 31; Arg at position 40; Leu at position 52; His at position 65; Ser at position 78; Asp at position 80; Tyr at position 87; Lys at position 120; Asp at position 122; Lys at position 129; Met at position 135; Phe at position 150; Ile at position 160; Ala at position 164; His at position 172; Phe at position 174; Leu at position 176; Asn at position 178; Asn at position 186; Glu at position 194; Gly at position 196; Thr at position 199; Ala at position 210; His at position 212; Arg at position 219; Pro at position 234; Asn at position 241; Leu at position 244; Thr at position 250; Ala at position 254; Tyr at position 265; Arg at position 266; Glu at position 273; Lys at position 275; Ser at position 276; an amino acid deletion at position 276; and Thr at position 279, wherein the position number corresponds to that of the human B7-1 amino acid sequence (SEQ ID NO:278).

82. The isolated or recombinant polypeptide of claim 80, wherein said polypeptide comprises an amino acid sequence having at least about 98% identity to at least one of SEQ ID NOS:69-92, 222-252, and 286-289, said amino acid sequence comprising at least one of:

Gly at position 2; Gly at position 8; Cys at position 27; His at position 65; Asp at position 80; Asp at position 122; Met at position 135; Phe at position 150; Ala at position 164; Phe at position 174; Asn at position 186; Glu at position 194; Arg at position 219; Thr at position 250; Arg at position 266; Lys at position 275; and Ser at position 276, wherein the amino acid position numbers correspond to that of the human B7-1 amino acid sequence (SEQ ID NO:278).

83. The isolated or recombinant polypeptide of claim 80, wherein said polypeptide comprises an amino acid sequence having at least about 98% identity to an extracellular domain of at least one of SEQ ID NOS:69-92, 222-252, and 286-289, said amino acid sequence comprising at least one of:

1000000000000000

His at position 65; Asp at position 80; Asp at position 122; Met at position 135; Phe at position 150; Ala at position 164; Phe at position 174; Asn at position 186; Glu at position 194; and Arg at position 219, wherein the amino acid position numbers correspond to that of the human B7-1 amino acid sequence (SEQ ID NO:278).

5

84. The isolated or recombinant polypeptide of claim 83, wherein said polypeptide comprises an amino acid sequence having at least about 98% identity to an extracellular domain of at least one of SEQ ID NOS:69-92, 222-252, 286-289, said sequence comprising at least two of :

10

His at position 65; Asp at position 80; Asp at position 122; Met at position 135; Phe at position 150; Ala at position 164; Phe at position 174; Asn at position 186; Glu at position 194; and Arg at position 219, wherein the amino acid position numbers correspond to that of the human B7-1 amino acid sequence (SEQ ID NO:278).

15

85. The isolated or recombinant polypeptide of claim 84, wherein said polypeptide comprises an extracellular domain of any one of SEQ ID NOS:81, 85, 86, 88, 90, and 91.

20

86. The isolated or recombinant polypeptide of claim 80, which polypeptide comprises an extracellular domain of any one of SEQ ID NOS:69-92, 222-252, and 286-289.

25

87. The isolated or recombinant polypeptide of claim 80, which polypeptide comprises an amino acid sequence of any one of SEQ ID NOS:69-92, 222-252, and 286-289.

88. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

30

89. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide has either a same binding affinity or an enhanced binding affinity for CTLA-4 as compared to a binding affinity of a wild type co-stimulatory molecule for CTLA-4.

90. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide has a decreased or a lowered binding affinity for CD28 as compared to a binding affinity of a wild type co-stimulatory molecule for CD28.

5 91. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide inhibits T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

10 92. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide inhibits T-cell proliferation.

15 93. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide induces a T-cell response less than that of human B7-1.

15 94. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

20 95. The isolated or recombinant polypeptide of claim 84 or 91, which polypeptide comprises an extracellular domain amino acid sequence of any one of SEQ ID NOS:69-92 and 222-247.

25 96. The isolated or recombinant polypeptide of claim 80 or 91, which polypeptide comprises an extracellular domain amino acid sequence encoded by a coding polynucleotide sequence, the coding polynucleotide sequence selected from the group:

(a) an extracellular domain coding sequence of a polynucleotide sequence selected from any of SEQ ID NOS:22-45 and 143-173;

(b) a polynucleotide sequence that encodes the extracellular domain of a polypeptide selected from any of SEQ ID NOS:69-92, 222-252, and 286-289; and

30 (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a polynucleotide sequence (a) or (b).

100-921-12252

97. An isolated or recombinant polypeptide comprising an amino acid sequence that differs from a primate B7-1 amino acid sequence in at least one mutation selected from:

5 Ser 12 Pro; Leu 25 Met; Gly 27 Cys; Ser 29 Pro; Lys 40 Arg; His 52 Leu; Tyr 65 His; Glu 122 Asp; Glu 129 Lys; Thr 135 Met; Thr 164 Ala; Ser 174 Phe; Glu 196 Gly; Ala 199 Thr; Thr 210 Ala; Lys 219 Arg; Thr 234 Pro; Asp 241 Asn; Val 254 Ala; Arg 275 Lys; Arg 276 Ser; or Arg 279 Thr; the mutation being indicated comprising a mutation relative to human B7-1 with the amino acid sequence shown in SEQ ID NO:278,

wherein said amino acid sequence does not occur in nature, and wherein said

10 polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

98. The isolated or recombinant polypeptide of claim 97, wherein said amino acid sequence differs from said primate B7-1 sequence in at least two of said mutations.

15 99. The isolated or recombinant polypeptide of claim 97, wherein said primate B7-1 is human B7-1 (SEQ ID NO:278).

100. The isolated or recombinant polypeptide of claim 99, wherein said 20 sequence differs from the human B7-1 sequence in at least two of said mutations.

101. An isolated or recombinant polypeptide comprising an amino acid sequence, said amino acid sequence having at least about 75% identity to at least one polypeptide sequence of SEQ ID NOS:263-272, or a subsequence thereof comprising the 25 extracellular domain, wherein said amino acid sequence is not a naturally-occurring amino acid sequence, and

wherein said polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

30 102. An isolated or recombinant polypeptide, which polypeptide comprises a non naturally-occurring amino acid sequence encoded by a nucleic acid comprising a polynucleotide sequence selected from:

- (a) a polynucleotide sequence selected from SEQ ID NOS:22-45, 143-173, 253-262, or a complementary polynucleotide sequence thereof;
- (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS:69-92, 222-247, 263-272, 286-289, or a complementary polynucleotide sequence thereof;
- 5 (c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b);
- (d) a polynucleotide sequence comprising all or a nucleotide fragment of (a),
10 (b), or (c), wherein the fragment encodes a polypeptide having a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1;
- (e) a polynucleotide sequence encoding a polypeptide comprising an amino acid sequence that is substantially identical over at least about 150 contiguous amino acid
15 residues of any one of SEQ ID NOS:69-92, 222-247, 263-272, 286-289, and
- (f) a polynucleotide sequence encoding a polypeptide that has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, which polynucleotide sequence has at least about 70% identity to at least one polynucleotide sequence of (a), (b), (c), or (d).
- 20

103. The isolated or recombinant polypeptide of claim 102, the polypeptide comprising an amino acid sequence of any one of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289.

25 104. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

30 105. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide inhibits T-cell proliferation.

106. The isolated or recombinant polypeptide of claim 102, wherein the polypeptide induces a T-cell response less than that of human B7-1.

TOKUYAMA 2020

107. An isolated or recombinant polypeptide comprising an amino acid sequence according to the formula:

MGHTRRQGTSP-X12-KCPYLKFFQLLV-X25-ACL-X29-

5 HLCGVIHVT-X40-EVKEVATLSCGLNVSVEELAQTRIHWQKEKKMVLTM
MSGDMNIWPEYKNRTIFDITNNLSIVILALRPSDEGYECVVLKY-X122-KDAFKR-
X129-HLAEVMLSVKAD FPTPSITDFEIPPSNIRRIICS-X164-
SGGFPEPHLFWLENGEELNAINTTVSQDPET-X196-LYTSSKLDNFNM
TANHSFMCLI-X219-YGHLRVNQTFNWNTPKQEHP-X241-NLLPSWA
10 ITLISANGIFVICCLTYRFAPRCRERKSNETLRRESVCPV (SEQ ID NO:287), or a subsequence thereof comprising the extracellular domain,
wherein position X12 is Ser or Pro; position X25 is Leu or Met; position X29 is Ser or Pro; position X40 is Lys or Arg; position X122 is Glu or Asp; position X129 is Glu or Lys; position X164 is Thr or Ala; position X196 is Glu or Gly; position X219 is Lys or
15 Arg; and position X241 is Asp or Asn.

108. The isolated or recombinant polypeptide of claim 107, which polypeptide comprises the extracellular domain of SEQ ID NO:288 or SEQ ID NO:289.

20 109. The isolated or recombinant polypeptide of claim 107, comprising the sequence SEQ ID NO:288 or SEQ ID NO:289.

25 110. The isolated or recombinant polypeptide of claim 107, wherein the polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1.

111. The isolated or recombinant polypeptide of claim 107 or 110, wherein the polypeptide inhibits T-cell proliferation.

30 112. The isolated or recombinant polypeptide of claim 107 or 110, wherein the polypeptide induces a T-cell response less than that of human B7-1.

13. An isolated or recombinant polypeptide comprising a subsequence of an amino acid sequence set forth in any of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289, wherein the subsequence is the extracellular domain of said amino acid sequence.

5 114. The isolated or recombinant polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a signal sequence.

115. The polypeptide of claim 114, wherein the signal sequence is selected from the signal sequence set forth in any of SEQ ID NOS:69-92, 222-247, 263-272, and 10 286-289.

116. The polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a transmembrane domain sequence or a cytoplasmic domain sequence, selected from the transmembrane domain sequence or the cytoplasmic domain sequence set forth in any of 15 SEQ ID NOS:69-92, 222-247, 263-272, and 286-289.

117. The polypeptide of claim 80, 97, 101, 102, 107, or 113 comprising a soluble extracellular domain.

20 118. The polypeptide of claim 80, 97, 101, 102, 107, or 113, wherein the polypeptide comprises a fusion protein comprising at least one additional amino acid sequence.

119. The polypeptide of claim 118, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.

25 120. The polypeptide of claim 119, wherein the at least one Ig polypeptide comprises a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

30 121. The polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a polypeptide purification subsequence.

122. The polypeptide of claim 121, wherein the polypeptide purification subsequence is selected from: an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion.

5 123. The polypeptide of claim 80, 97, 101, 102, 107, or 113, comprising a modified amino acid.

10 124. The polypeptide of claim 123, wherein the modified amino acid is selected from the group consisting of: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

15 125. A composition comprising at least one polypeptide of claim 124 and a pharmaceutically acceptable excipient.

126. A composition comprising at least one polypeptide of claim 80, 97, 101, 102, 107, or 113, and a pharmaceutically acceptable excipient.

20 127. A composition comprising:
an isolated or recombinant polypeptide comprising the amino acid sequence of SEQ ID NOS:69-92, 222-247, 263-272, 286-289, or a costimulatory fragment thereof, wherein said costimulatory fragment has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, and a carrier.

25 128. An isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from:

(a) a polynucleotide sequence selected from SEQ ID NOS:22-45, 143-173, or a complementary polynucleotide sequence thereof;

30 (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NOS:69-92, 222-247, 286-289, or a complementary polynucleotide sequence thereof;

(c) a polynucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b); and

(d) a polynucleotide sequence comprising all or a nucleotide fragment of (a),
(b), or (c);

wherein (c) or (d) encodes a polypeptide having a non naturally-occurring
amino acid sequence comprising at least one of:

5 Gly at position 2; Thr at position 4; Arg at position 5; Gly at position 8; Pro
at position 12; Met at position 25; Cys at position 27; Pro at position 29; Leu at position 31;
Arg at position 40; Leu at position 52; His at position 65; Ser at position 78; Asp at position
80; Tyr at position 87; Lys at position 120; Asp at position 122; Lys at position 129; Met at
position 135; Phe at position 150; Ile at position 160; Ala at position 164; His at position
10 172; Phe at position 174; Leu at position 176; Asn at position 178; Asn at position 186; Glu
at position 194; Gly at position 196; Thr at position 199; Ala at position 210; His at position
212; Arg at position 219; Pro at position 234; Asn at position 241; Leu at position 244; Thr
at position 250; Ala at position 254; Tyr at position 265; Arg at position 266; Glu at
position 273; Lys at position 275; Ser at position 276; an amino acid deletion at position
15 276; and Thr at position 279, wherein the number of the amino acid position corresponds to
that of the human B7-1 amino acid sequence (SEQ ID NO:278), and wherein said
polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the
CTLA-4/CD28 binding affinity ratio of human B7-1 and/or an ability to induce a T cell
proliferation response that is about equal to or greater than that induced by human B7-1.

20 129. An isolated or recombinant nucleic acid comprising a polynucleotide
sequence selected from:

(a) a polynucleotide sequence selected from SEQ ID NOS:253-262, or a
complementary polynucleotide sequence thereof;

25 (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID
NOS:263-272, or a complementary polynucleotide sequence thereof;

(c) a polynucleotide sequence which, but for the degeneracy of the genetic
code, hybridizes under highly stringent conditions over substantially the entire length of
polynucleotide sequence (a) or (b) and encodes a polypeptide having a non naturally-
occurring sequence; and

30 (d) a polynucleotide sequence comprising all or a nucleotide fragment of (a),
(b), or (c), wherein the fragment encodes a polypeptide having (i) a non naturally-occurring

1003234-1222004

sequence and (ii) a CTLA-4/CD28 binding affinity ratio about equal to or greater than the
CTLA-4/CD28 binding affinity ratio of human B7-1.

130. An isolated or recombinant nucleic acid comprising a polynucleotide
5 sequence encoding a polypeptide, the encoded polypeptide comprising an amino acid
sequence which is substantially identical over at least about 150 contiguous amino acid
residues of any one of SEQ ID NOS:69-92, 222-247, 263-272, and 286-289.

131. The nucleic acid of claim 44, wherein the encoded polypeptide is
10 substantially identical over at least about 200 contiguous amino acid residues of any one of
SEQ ID NOS:69-92, 222-247, 263-272, and 286-289.

132. An isolated or recombinant nucleic acid comprising a nucleotide
sequence coding for a polypeptide comprising the amino acid sequence set forth in any of
15 SEQ ID NOS:69-92, 222-247, 263-272, and 286-289, or a subsequence thereof, wherein the
subsequence comprises at least one of: the signal peptide sequence of said polypeptide, the
extracellular domain of said polypeptide, the transmembrane domain of said polypeptide,
and the cytoplasmic domain of said polypeptide, and wherein the amino acid sequence or
subsequence is a non naturally-occurring amino acid sequence.
20

133. The nucleic acid of claim 128, 129, 130, or 132, wherein the
polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the
CTLA-4/CD28 binding affinity ratio of human B7-1.

134. The nucleic acid of claim 128, 129, 130, or 132, wherein the
polypeptide has either a same binding affinity or an enhanced binding affinity for CD28 as
compared to a binding affinity of a wild type co-stimulatory molecule for CD28.
25

135. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded
30 polypeptide has a decreased or a lowered binding affinity for CTLA-4 as compared to a
binding affinity of a wild type co-stimulatory molecule for CTLA-4.

136. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide inhibits T-cell proliferation or T-cell activation or both T-cell proliferation and T-cell activation.

5 137. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide modulates T-cell activation, but does not induce proliferation of purified T-cells activated by soluble anti-CD3 mAbs.

10 138. The nucleic acid of claim 128, 129, 130, or 132, wherein the nucleic acid encodes a fusion protein comprising at least one additional amino acid sequence.

139. The nucleic acid of claim 138, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.

15 140. The nucleic acid of claim 139, wherein the at least one Ig polypeptide comprises a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3 domain.

20 141. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide comprises a signal peptide sequence.

142. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide comprises a precursor peptide.

25 143. The nucleic acid of claim 128, 129, 130, or 132, wherein the encoded polypeptide comprises an epitope tag sequence.

144. A cell comprising the nucleic acid of claim 128, 129, 130, or 132.

30 145. The cell of claim 144, wherein the cell expresses a polypeptide encoded by the nucleic acid.

146. A vector comprising the nucleic acid of claim 128, 129, 130, 132,
133, or 136.

147. The vector of claim 146, wherein the vector comprises a plasmid, a
5 cosmid, a phage, a virus, a virus-like particle, or a fragment of a virus.

148. The vector of claim 146, wherein the vector is an expression vector.

149. The expression vector of claim 148, wherein the nucleic acid is
10 operably linked to a promoter.

150. The expression vector of claim 149, further comprising a
polynucleotide sequence encoding at least one Ig polypeptide or fragment thereof.

15 151. The expression vector of claim 150, wherein the at least one Ig
polypeptide is a human IgG polypeptide comprising an Fc hinge, a CH2 domain, and a CH3
domain.

20 152. The expression vector of claim 150, wherein the promoter is a CMV
promoter.

153. The expression vector of claim 150, further comprising a BGH polyA
sequence.

25 154. A host cell comprising the vector of claim 146.

155. A composition comprising the nucleic acid of claim 128, 129, 130, or
132, and an excipient.

30 156. The composition of claim 155, wherein the excipient is a
pharmaceutically acceptable excipient.

157. A composition of matter comprising at least one nucleic acid of claim
128, 129, 130, 132, 133, or 136.

158. The composition of claim 157, wherein the composition comprises a
5 library comprising at least about 2, 5, 10, 50 or more nucleic acids.

159. A composition produced by cleaving at least one nucleic acid of
claim 128, 129, 130, or 132.

10 160. The composition of claim 159, wherein the cleaving comprises
mechanical, chemical, or enzymatic cleavage.

161. The composition of claim 160, wherein the enzymatic cleavage
comprises cleavage with a restriction endonuclease, an RNase, or a DNase.

15 162. A composition produced by a process comprising incubating at least
one nucleic acid of claim 128, 129, 130, or 132 in the presence of deoxyribonucleotide
triphosphates and a nucleic acid polymerase.

20 163. The composition of claim 162, wherein the nucleic acid polymerase
is a thermostable polymerase.

164. An isolated or recombinant nucleic acid encoding a polypeptide that
has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28
25 binding affinity ratio of human B7-1, produced by mutating or recombining at least one
nucleic acid of claim 128, 129, 130, or 132.

165. An isolated or recombinant polypeptide comprising an amino acid
sequence according to the formula:

30 MGHTMKWGS~~L~~PPKR~~P~~CLWLSQLLVLTGLFYFC~~S~~G~~I~~TPK
SVTKRVKETVM-X50-SCDY-X55-X56-STEELTS~~L~~R~~I~~Y~~W~~QKDSKMVL
AILPGKVQVWPEYKNRTITD MNDNPRIVILALRLSD-X113-GTYTCV-X120-QK-
X123-X124-X125-X126-G-X128-X129-X130-X131-EHL-X135-SV-X138-L-X140-

IRADFPVPSITDIGHPAPNVK RIRCSASG-X170-FPEPRLAWMEDGEEL NAVNTTV-
X193-X194-X195-LDTELYSVSSELD-X209-N-X211-
TNNHSIVCLIKYGELSVSQIFPWSKPK QEPPIDQLPFWVI-X252-X253-
VSGALVLTAVVLYCLACRHVAR (SEQ ID NO:290), or a subsequence thereof

5 comprising the extracellular domain,

wherein position X50 is Leu or Pro; position X55 is Asn or Ser; position X56 is Ala or Thr; position X113 is Ser or Lys; position X120 is Ile or Val; position X123 is Pro or deleted; position X124 is Val, Asn, or Asp; position X125 is Leu or Glu; position X126 is Lys or Asn; position X128 is Ala or Ser; position X129 is Tyr or Phe; position X130 is Lys or Arg; position X131 is Leu or Arg; position X135 is Ala or Thr; position X138 is Arg or Thr; position X140 is Met or Ser; position X170 is Asp or Gly; position X193 is Asp or is deleted; position X194 is Gln or is deleted; position X195 is Asp or is deleted; position X211 is Val or Ala; position X252 is Ile or Val; and position X253 is Leu or Pro.

15 166. The isolated or recombinant polypeptide of claim 165, which polypeptide comprises an amino acid sequence of any one of SEQ ID NOS:59, 62, 180, 184, 188, 195, 196, 200, 201, 204, 211, 213, 219, and 291.

20 167. An isolated or recombinant polypeptide comprising an amino acid sequence according to the formula:

MGHTMKWG-X9-LPPKRpclwlsqllvltglfyfcsG-X35-
TPKSVTKRV KETVMLSCDY-X55-TSTEELTSRlYwQKDskMVLAILPGKVQVW
PEYKNRTITDMNDNPRIVILALR-X110-SDSGTYTCVIQKP-X124-LKGAYKLEHL-
X135-SVRLMIRADFPVPTINDGNPSPNIRRLICSTSGGFPRPHLYWLENG-X183-
25 ELNATNTT-X192-SQDPETKLYMISSELDFN-X211-TSN-X215-X216-X217-
LCLVKYGDLTvSQ-X231-FYWQESKPTPSANQHLTWTIIIPVSAFGISVIIAVI
LTCLTCRNAAIRQRRENEV-X288-M-X290-SCSQSP (SEQ ID NO:292), or a subsequence thereof comprising the extracellular domain,

wherein position X9 is Thr or Ser; position X35 is Ile or Thr; position X55 is Asn or Ser; position X110 is Leu or Pro; position X124 is Asp or Val; position X135 is Thr or Ala; position X183 is Lys or Glu; position X192 is Leu or Val; position X211 is Met or Thr; position X215 is His or is deleted; position X216 is Ser or is deleted; position X217 is

Phe or is deleted; position X231 is Thr or Ser; position X288 is Lys or Glu; position X290 is Glu or Gln, and wherein said sequence is a non naturally-occurring sequence.

168. The isolated or recombinant polypeptide of claim 167, which
5 polypeptide comprises an amino acid sequence of any one of SEQ ID NOS:48, 182, 183,
212, 214, 216, 218, 221, and 293.

169. An isolated or recombinant polypeptide comprising the sequence SEQ
ID NO:93, SEQ ID NO:94, or a subsequence thereof, wherein the subsequence comprises at
10 least one of: the signal peptide of said polypeptide, the extracellular domain of said
polypeptide, the transmembrane domain of said polypeptide, and the cytoplasmic domain of
said polypeptide.

170. An isolated or recombinant nucleic acid comprising a polynucleotide
15 sequence selected from:

(a) a polynucleotide sequence selected from SEQ ID NO:46, SEQ ID NO:47,
or a complementary polynucleotide sequence thereof;

(b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID
NO:93, SEQ ID NO:94, or a complementary polynucleotide sequence thereof;

20 (c) a polynucleotide sequence encoding a subsequence of a polypeptide
selected from SEQ ID NO:93, SEQ ID NO:94, or a complementary polynucleotide
sequence thereof, wherein the subsequence comprises at least one of: the signal peptide of
said polypeptide, the extracellular domain of said polypeptide, the transmembrane domain
of said polypeptide, and the cytoplasmic domain of said polypeptide.

25 171. A polypeptide which is specifically bound by a polyclonal antisera
raised against at least one antigen, the at least one antigen comprising the sequence SEQ ID
NOS:48-94, 174-252, 263-272, 283-293, or a fragment thereof, wherein the antisera is
subtracted with a polypeptide encoded by one or more of GenBank Nucleotide Accession
30 Nos: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153,
AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824,
AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833,
AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762,

PCT/EP2007/000244

AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

- 5 172. An antibody or antisera produced by administering the polypeptide of
claim 1, 80, 101, or 169 to a mammal, which antibody or antisera specifically binds at least
one antigen, the at least one antigen comprising a polypeptide comprising one or more of
the amino acid sequences SEQ ID NOS:48-94, 174-252, 263-272, and 283-293, which
antibody or antisera does not specifically bind to a polypeptide encoded by one or more of
10 GenBank Nucleotide Accession Nos: A92749, A92750, AA983817, AB026121,
AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895,
AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830,
AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747,
AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587,
15 M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695,
S74696, U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622,
X60958, Y08823, and Y09950.

- 20 173. An antibody or antisera which specifically binds a polypeptide, the
polypeptide comprising an amino acid sequence selected from: SEQ ID NOS:48-94, 174-
252, 263-272, and 283-293, wherein the antibody or antisera does not specifically bind to a
polypeptide encoded by one or more of GenBank Nucleotide Accession Nos: A92749,
A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465,
AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825,
25 AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834,
AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904,
AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077,
NM005191, S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840,
U26832, U33063, U33208, U57755, U88622, X60958, Y08823, and Y09950.

30

174. A method of producing a polypeptide, the method comprising:

(a) introducing into a population of cells a nucleic acid of claim 43, 46, 128, 129, 132, or 170, the nucleic acid operatively linked to a regulatory sequence effective to produce the encoded polypeptide;

- (b) culturing the cells in a culture medium to produce the polypeptide; and
(c) isolating the polypeptide from the cells or from the culture medium.

5

175. A method of producing a polypeptide, the method comprising
(a) introducing into a population of cells a recombinant expression vector comprising the nucleic acid of claim 43, 46, 128, 129, 132, or 170;

10

(b) culturing the cells in a culture medium to produce the polypeptide encoded by the expression vector, and

- (c) isolating the polypeptide from the cells or from the culture medium.

15

176. A method of producing a polypeptide, the method comprising:

(a) introducing into a population of cells a recombinant expression vector

comprising the nucleic acid of claim 43, 46, 128, 129, 132, or 170;

(b) administering the expression vector into a mammal; and

(c) isolating the polypeptide from the mammal or from a byproduct of the mammal.

20

177. A method of inducing T-cell proliferation, the method comprising:
contacting a population of Tcells with a polypeptide of claim 1, 80, 101, or 169, thereby
inducing proliferation of the T cells.

25

178. A method of inhibiting T-cell proliferation, the method comprising:
contacting a population of T cells with a polypeptide of claim 1, 80, 101, or 169, thereby
inhibiting proliferation of the T cells.

179. A method of modifying T-cell proliferation, the method comprising:

30 contacting a population of T cells with a polypeptide of claim 1, 80, 101, or 169, thereby
modifying proliferation of the T cells.

180. A method of modifying T-cell activation, the method comprising:
contacting a population of T cells with a polypeptide of claim 1, 80, 101, or 169, thereby
modifying activation of the T cells.

5 181. The method of claim 177, 178, 179, or 180 wherein the T cells are in
culture.

10 182. A method of treating an autoimmune disorder or medical condition in
a subject, the method comprising: administering to the subject an effective amount of the
polypeptide of claim 1, 80, 101, or 169.

15 183. A method of treating an autoimmune disorder or medical condition in
a subject, the method comprising: administering to the subject an effective amount of an
expression vector comprising the nucleic acid of claim 43, 46, 128, 129, 132, or 170

184. The method of claim 182, wherein the autoimmune disorder or
medical condition is selected from the group comprising: multiple sclerosis, rheumatoid
arthritis, lupus erythematosus, psoriasis, and type I diabetes.

20 185. The method of claim 182, wherein the medical condition comprises
an allogeneic or xenogeneic graft or transplant.

25 186. A method of treating a medical disorder in a subject, the method
comprising: administering to the subject an effective amount of the polypeptide of claim 1,
80, 101, or 169.

187. The method of claim 186, wherein the medical disorder comprises:
cancer, viral infection (e.g. HIV), or bacterial infection.

30 188. In a method of treating a disorder treatable by administration of a co-
stimulatory molecule to a subject, an improved method comprising: administering to the
subject an effective amount of the polypeptide of claim 1, 80, 101, or 169.

189. The method of claim 188, wherein the disorder treatable by administration of a co-stimulatory molecule is selected from the group comprising: sclerosis, rheumatoid arthritis, lupus erythematosus, psoriasis, type I diabetes, allogeneic grafts, xenogeneic grafts, cancer, viral infection, and bacterial infection.

5

190. A method of recombination, the method comprising recursively recombining one or more nucleic acids of claim 43, 46, 128, 129, 132, or 170, with at least one additional nucleic acid.

10

191. The method of claim 190, wherein the at least one additional nucleic acid encodes a co-stimulatory homologue or subsequence thereof.

15

192. The method of claim 190, wherein the recursive recombination produces at least one library of recombinant co-stimulatory homologue nucleic acids.

20

193. A nucleic acid library produced by the method of claim 192.

194. A population of cells comprising the library of claim 193.

195. A recombinant co-stimulatory homologue nucleic acid produced by the method of claim 191.

25

196. A cell comprising the nucleic acid of claim 195.

197. The method of claim 190, wherein the recursive recombination is performed in vitro.

30

198. The method of claim 190, wherein the recursive recombination is performed in vivo.

199. A method of producing a modified co-stimulatory nucleic acid homologue comprising mutating a nucleic acid of claim 43, 46, 128, 129, 132, or 170.

200. The modified co-stimulatory homologue nucleic acid homologue produced by the method of claim 199.

201. A computer or computer readable medium comprising a database 5 comprising a sequence record comprising one or more character strings corresponding to a nucleic acid or polypeptide sequence selected from SEQ ID NOS:1-272 and 283-293.

202. An integrated system comprising a computer or computer readable medium comprising a database comprising one or more sequence records, each of said one 10 or more sequence records comprising one or more character strings corresponding to a nucleic acid or polypeptide sequence selected from SEQ ID NOS:1-272 and 283-293, the integrated system further comprising a user input interface allowing a user to selectively view one or more sequence records.

203. The integrated system of claim 202, the computer or computer readable medium comprising an alignment instruction set which aligns the one or more character strings with one or more additional character strings corresponding to a nucleic acid or polypeptide sequence. 15

204. The integrated system of claim 203, wherein the instruction set 20 comprises one or more of: a local homology comparison determination, a homology alignment determination, a search for similarity determination, and a BLAST determination.

205. The integrated system of claim 203, further comprising a user 25 readable output element which displays an alignment produced by the alignment instruction set.

206. The integrated system of claim 202, the computer or computer readable medium further comprising an instruction set which translates one or more nucleic 30 acid sequences, each of said one or more nucleic acid sequences comprising a sequence selected from SEQ ID NOS:1-47, 95-173, and 253-262, into an amino acid sequence.

100-322-14-122004

207. The integrated system of claim 202, the computer or computer readable medium further comprising an instruction set for reverse-translating at least one amino acid sequence comprising a sequence selected from SEQ ID NOS:48-94, 174-252, 263-272, and 283-293, into a nucleic acid sequence.

5

208. The integrated system of claim 207, wherein the instruction set selects the nucleic acid sequence by applying a codon usage instruction set or an instruction set which determines sequence identity to a test nucleic acid sequence.

10

209. A method of using a computer system to present information pertaining to at least one of a plurality of sequence records stored in a database, said sequence records each comprising one or more character strings corresponding to SEQ ID NOS:1-272 and 283-293, the method comprising:

15

(a) determining a list of one or more character strings corresponding to one or more of SEQ ID NOS:1-272 and 283-293, or a subsequence thereof;
(b) determining which character strings of said list are selected by a user; and
(c) displaying the selected character strings, or aligning the selected character strings with an additional character string.

20

210. The method of claim 209, further comprising displaying an alignment of the selected character string with the additional character string.

25

211. The method of claim 209, further comprising displaying the list.

30

212. A nucleic acid which comprises a unique subsequence in a nucleic acid selected from SEQ ID NOS:1-47, 95-173, and 253-262, wherein the unique subsequence is unique as compared to a nucleic acid corresponding to any of GenBank Nucleotide Accession No.: A92749, A92750, AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696,

100032214-122001
U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958,
Y08823, and Y09950.

213. A polypeptide which comprises a unique subsequence in a
5 polypeptide selected from: SEQ ID NOS:48-94, 174-252, 263-272, and 283-293, wherein
the unique subsequence is unique as compared to a polypeptide encoded by any of GenBank
Nucleotide Accession Nos: A92749, A92750, AA983817, AB026121, AB030650,
AB030651, AB038153, AF010465, AF065893, AF065894, AF065895, AF065896,
AF079519, AF106824, AF106825, AF106828, AF106829, AF106830, AF106831,
10 AF106832, AF106833, AF106834, AF203442, AF203443, AF216747, AF257653,
AH004645, AH008762, AX000904, AX000905, D49843, L12586, L12587, M27533,
M83073, M83074, M83075, M83077, NM005191, S74541, S74540, S74695, S74696,
U05593, U10925, U19833, U19840, U26832, U33063, U33208, U57755, U88622, X60958,
Y08823, and Y09950.
15

214. A target nucleic acid which, but for the degeneracy of the genetic
code, hybridizes under stringent conditions to a unique coding oligonucleotide which
encodes a unique subsequence in a polypeptide selected from: SEQ ID NOS:48-94, 174-
252, 263-272, and 283-293, wherein the unique subsequence is unique as compared to a
20 polypeptide encoded by any of GenBank Nucleotide Accession No.: A92749, A92750,
AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893,
AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828,
AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442,
AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905,
25 D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191,
S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063,
U33208, U57755, U88622, X60958, Y08823, and Y09950.

215. The nucleic acid of claim 214, wherein the stringent conditions are
30 selected such that a perfectly complementary oligonucleotide to the coding oligonucleotide
hybridizes to the coding oligonucleotide with at least about a 5x higher signal to noise ratio
than for hybridization of the perfectly complementary oligonucleotide to a control nucleic
acid corresponding to any of GenBank Nucleotide Accession No.: A92749, A92750,

10032214-122004

AA983817, AB026121, AB030650, AB030651, AB038153, AF010465, AF065893,
AF065894, AF065895, AF065896, AF079519, AF106824, AF106825, AF106828,
AF106829, AF106830, AF106831, AF106832, AF106833, AF106834, AF203442,
AF203443, AF216747, AF257653, AH004645, AH008762, AX000904, AX000905,
5 D49843, L12586, L12587, M27533, M83073, M83074, M83075, M83077, NM005191,
S74541, S74540, S74695, S74696, U05593, U10925, U19833, U19840, U26832, U33063,
U33208, U57755, U88622, X60958, Y08823, and Y09950, wherein the target nucleic acid
hybridizes to the unique coding oligonucleotide with at least about a 2x higher signal to
noise ratio as compared to hybridization of the control nucleic acid to the coding
10 oligonucleotide.

216. A method of therapeutic or prophylactic treatment of a disease or disorder in a subject in need of such treatment, comprising: administering to the subject at least one polypeptide of claim 1, 80, 101, or 169 or at least one nucleic acid of claim 43, 46, 15 128, 129, 132, or 170, and at least one immunogen specific for said disease or disorder, wherein the combined amount of the at least one polypeptide or at least one nucleic acid and the at least one immunogen is effective to prophylactically or therapeutically treat said disease or disorder.

20 217. The method of claim 216, wherein the at least one polypeptide or nucleic acid is present in an amount sufficient to enhance, diminish, modulate or modify an immune response induced by the at least one immunogen.

25 218. The method of claim 216, wherein a composition comprising the at least one polypeptide or nucleic acid, the immunogen, and a pharmaceutically acceptable excipient is administered to the subject in an amount effective to treat said disease or disorder.

30 219. The method of claim 216, wherein the subject is a mammal.

220. The method of claim 219, wherein the mammal is a human.

10032214-133001
221. The method of claim 216, wherein the polypeptide is administered in vivo to the subject.

5 222. The method of claim 216, wherein the polypeptide is administered in vitro or ex vivo to one or more cells of the subject.

10 223. A method of modulating an immune response in a subject, comprising: administering to the subject a polynucleotide comprising a nucleic acid sequence of claim 43, 46, 128, 129, 132, or 170, operably linked to a promoter sequence that controls the expression of said nucleic acid sequence, said polynucleotide being present in an amount sufficient that uptake of said polynucleotide into one or more cells of the subject occurs and sufficient expression of said nucleic acid sequence results to produce an amount of a polypeptide effective to modulate an immune response.

15 224. The method of claim 223, further comprising administering to the subject an antigen specific for the disease or disorder, wherein the polynucleotide is administered to the subject in an amount sufficient to modulate the immune response induced in the subject by the antigen.

20 225. The method of claim 223, wherein the polynucleotide further comprises a nucleotide sequence encoding for an antigen.

25 226. The method of claim 223, wherein the polynucleotide further comprises at least one additional nucleotide sequence encoding a cytokine, adjuvant, co-stimulatory molecule, or at least one additional nucleotide sequence comprising a promoter.

227. The method of claim 223, wherein the subject is a mammal.

228. The method of claim 227, wherein the mammal is a human.

30 229. The method of claim 223, wherein said polynucleotide comprises a vector.

10032244-122001

230. A method of treating a disease or disorder in a subject in need of such treatment, comprising: administering to the subject at least one polypeptide of claim 1, 80, 101, or 169 or at least one nucleic acid of claim 43, 46, 128, 129, 132, or 170 in an amount effective to treat said disease or disorder.

5

231. A method of therapeutic or prophylactic treatment of a disease or disorder in a subject in need of such treatment, comprising: administering to the subject a polypeptide of claim 32 or 117 and an immunogen specific for said disease or disorder, wherein the combined amount of polypeptide and immunogen is effective to prophylactically or therapeutically treat said disease or disorder.

10

232. The method of claim 231, wherein the polypeptide is present in an amount sufficient to enhance, diminish or modify an immune response induced by the immunogen.

15

233. The method of claim 231, wherein a composition comprising the polypeptide, the immunogen, and a pharmaceutically acceptable excipient is administered to the subject in an amount effective to treat the disease or disorder.

20

234. The method of claim 231, wherein the subject is a mammal.

235. The method of claim 234, wherein the mammal is a human.

25

236. The method of claim 231, wherein the polypeptide is administered *in vivo* to the subject.

237. The method of claim 231, wherein the polypeptide is administered *in vitro* or *ex vivo* to one or more cells of the subject.

30

238. A method of treating a disease or disorder in a subject in need of such treatment, comprising: administering to the subject a polypeptide of claim 58 in an amount effective to treat the disease or disorder.

239. The isolated or recombinant polypeptide of claim 165, comprising three or more of:

Leu at position X50; Asn at position X55; Ala at position X56; Ser at position X113; Ile at position X120; Pro at position X123; Val at position X124; Leu at position X125; Lys at position X126; Ala at position X128; Tyr at position X129; Lys at position X130; Leu at position X131; Ala at position X135; Arg at position X138; Met at position X140; Asp at position X170; Asp at position X193; Asp at position X194; Asp at position X195; Val at position X211; Ile at position X252; and Leu at position X253.

10 240. The isolated or recombinant polypeptide of claim 167, comprising three or more of:

Thr at position X9; Ile at position X35; Asn at position X55; Leu at position X110; Asp at position X124; Thr at position X135; Lys at position X183; Leu at position X192; Met at position X211; His at position X215; Ser at position X216; Phe at position X217; Thr at position X231; Lys at position X288; and Glu at position X290.

15 241. A method of modulating or altering a T-cell response specific to an antigen in a subject, the method comprising administering to the subject at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS:48-94, 174-252, 263-272 and 283-293 or fragment thereof, and a polynucleotide sequence 20 encoding the antigen or antigenic fragment thereof, wherein each of the at least one polynucleotide sequences is expressed in the subject in an amount effective to modulate or alter a T cell response.

25 242. The method of claim 241, wherein the at least one polynucleotide sequence encoding a polypeptide comprises a polynucleotide sequence selected from any of SEQ ID NOS:1-47, 95-173, and 253-262.

243. The method of claim 241, wherein the polypeptide or fragment thereof interacts with or binds a T cell surface receptor.

244. The method of claim 241, wherein the T-cell response is enhanced.

30 245. The method of claim 244, wherein the enhanced T cell response is sufficient to eliminate cells bearing the antigen or antigenic fragment thereof.

246. The method of claim 241, wherein the T-cell response is suppressed or inhibited.

- P00022151422004
247. The method of claim 241, wherein the antigen or antigenic fragment thereof is an antigen or antigenic fragment thereof of an infectious agent or a cancer.
248. The method of claim 244, wherein the polypeptide comprises SEQ ID NO:66 or the extracellular domain amino acid sequence thereof.
- 5 249. The method of claim 245, wherein the polypeptide comprises SEQ ID NO:86 or the extracellular domain amino acid sequence thereof.
250. The method of claim 244, wherein the at least one polynucleotide sequence encoding a NCSM polypeptide or fragment thereof is operably linked to a promoter in a first vector.
- 10 251. The method of claim 250, wherein the at least one polynucleotide sequence encoding the antigen or antigenic fragment thereof is operably linked to a promoter in the first vector.
252. The method of claim 250, wherein the at least one polynucleotide sequence encoding the antigen or antigenic fragment thereof is operably linked to a promoter in the a second vector.
- 15 253. A vector comprising at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS:48-94, 174-252, 263-272 and 283-293 or fragment thereof, and a polynucleotide sequence encoding the antigen or antigenic fragment thereof, wherein the polypeptide or fragment thereof interacts with or binds to a T cell receptor when expressed in a subject, and wherein each of the at least one polynucleotide sequences is operably linked to a promoter for expression in the subject and is present in an amount sufficient that when expressed is effective to modulate or alter a T cell response.
- 20 254. The vector of claim 253, wherein the at least one polynucleotide sequence encoding a polypeptide comprises a polynucleotide sequence of any of SEQ ID NOS:1-47, 95-173, and 253-262.
- 25 255. The vector of claim 253, wherein each of the at least one polynucleotide sequences is expressed in the subject in an amount effective to enhance a T cell response such that cells expressing the antigen or antigenic fragment thereof are eliminated.
- 30 256. The vector of claim 253, wherein each of the at least one polynucleotide sequences is expressed in the subject in an amount effective to inhibit a T cell response.

40032215 1122001

257. A vector comprising at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS:48-94, 174-252, 263-272 and 283-293 or fragment thereof, wherein the polypeptide or fragment thereof interacts with or binds to a T cell receptor when expressed in a subject, wherein the at least one polynucleotide sequence is operably linked to a promoter for expression in the subject and is present in an amount sufficient that when expressed is effective to modulate or alter a T cell response.

5 258. A method of modulating or altering an immune response in a subject, the method comprising introducing into cells of a tumor of the subject at least one polynucleotide sequence encoding a polypeptide comprising any of SEQ ID NOS:48-94, 10 174-252, 263-272 and 283-293 or fragment thereof, wherein the polypeptide or fragment thereof interacts with or binds to a T cell receptor when expressed in a subject, and wherein the at least one polynucleotide sequence is operably linked to a promoter for expression in the subject and is present in an amount sufficient that when expressed is effective to modulate or alter a T cell response.

15 259. An isolated or recombinant polypeptide comprising an amino acid sequence corresponding to an extracellular domain, wherein said amino acid sequence has at least about 92% amino acid sequence identity to the amino acid sequence corresponding to the extracellular domain of SEQ ID NO:66, and wherein said polypeptide has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio 20 of human B7-1.

260. The isolated or recombinant polypeptide of claim 259, further comprising at least one further amino acid sequence corresponding to a signal peptide.

261. The isolated or recombinant polypeptide of claim 260, further comprising at least one further amino acid sequence corresponding to a transmembrane 25 domain or a cytoplasmic domain.

262. The isolated or recombinant polypeptide of claim 259, wherein said extracellular domain amino acid sequence has at least about 95% amino acid sequence identity to an extracellular domain amino acid sequence of SEQ ID NO:66, and wherein said polypeptide has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 30 binding affinity ratio of human B7-1, and/or an ability to induce a T cell proliferation response at least about equal to or greater than the T cell proliferation response induced by human B7-1.

263. An isolated or recombinant nucleic acid comprising a nucleotide sequence selected from the group of:

(a) a nucleotide sequence that encodes an extracellular domain (ECD), said nucleotide sequence comprising an ECD coding subsequence of a polynucleotide sequence

5 selected from the group of SEQ ID NOS:1-21 and 95-142, or a complementary nucleotide sequence thereof;

(b) a nucleotide sequence encoding an ECD, said ECD comprising an amino acid subsequence of a polypeptide sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary nucleotide sequence thereof; and

10 (c) a nucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b), wherein said nucleotide sequence encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1 and/or an ability to induce a T cell proliferation response equal to or greater than that induced by human B7-1.

264. The isolated or recombinant nucleic acid of claim 263, wherein the nucleotide sequence of (c) hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) and encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

265. The isolated or recombinant nucleic acid of claim 264, further comprising at least a second nucleotide sequence that encodes a signal peptide, wherein said second nucleotide sequence is selected from the group of:

25 (a) a nucleotide sequence comprising a signal peptide coding subsequence of a polynucleotide sequence selected from the group of SEQ ID NOS:1-21 and 95-142, or a complementary nucleotide sequence thereof;

(b) a nucleotide sequence encoding a signal peptide, said signal peptide comprising an amino acid subsequence of a polypeptide sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary nucleotide sequence thereof;

(c) a nucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of

polynucleotide sequence (a) or (b), wherein said nucleotide sequence encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1; and

- (d) a nucleotide sequence encoding a signal peptide of a B7-1 polypeptide.

5

266. The isolated or recombinant nucleic acid of claim 265, wherein the nucleotide sequence of (c) hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) and encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio greater than the CD28/CTLA-4 binding affinity ratio of human B7-1 or an ability to induce a T cell proliferation response about equal to or greater than that induced by human B7-1.

10

267. The isolated or recombinant nucleic acid of claim 265, further comprising at least a third nucleotide sequence encoding a transmembrane domain selected from the group of:

15

(a) a nucleotide sequence comprising a transmembrane domain coding subsequence of a polynucleotide sequence selected from the group of SEQ ID NOS:1-21 and 95-142, or a complementary nucleotide sequence thereof;

20

(b) a nucleotide sequence encoding a transmembrane domain, said transmembrane domain comprising an amino acid subsequence of a polypeptide sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary nucleotide sequence thereof;

25

(c) a nucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b), wherein said nucleotide sequence encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1; and

(d) a nucleotide sequence that encodes a transmembrane domain of a B7-1 polypeptide.

30

268. The isolated or recombinant nucleic acid of claim 267, further comprising at least a fourth nucleotide sequence encoding a cytoplasmic domain selected from the group of:

- 1003254-122004
- (a) a nucleotide sequence comprising a cytoplasmic domain coding subsequence of a polynucleotide sequence selected from the group of SEQ ID NOS:1-21 and 95-142, or a complementary nucleotide sequence thereof;
- (b) a nucleotide sequence encoding a cytoplasmic domain, said cytoplasmic domain comprising an amino acid subsequence of a polypeptide sequence selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, or a complementary nucleotide sequence thereof;
- (c) a nucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a polynucleotide sequence (a) or (b), wherein said nucleotide sequence encodes a polypeptide that has a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1; and
- (d) a nucleotide sequence that encodes a cytoplasmic domain of a B7-1 polypeptide.
269. An isolated or recombinant polypeptide variant comprising an amino acid sequence that differs from the amino acid sequence of a primate B7-1, wherein the difference between the amino acid sequence of the variant and the amino acid sequence of the primate B7-1 comprises a different amino acid at position 65 other than alanine, wherein the position corresponds to the position in the amino acid sequence of human B7-1 of SEQ ID NO:278.
270. The isolated or recombinant polypeptide variant of claim 269, wherein the different amino acid is selected from the group of His, Arg, Lys, Pro, Phe, and Trp.
271. The isolated or recombinant polypeptide variant of claim 270, wherein the primate B7-1 is human B7-1 and the different amino acid is histidine.
272. The isolated or recombinant polypeptide variant of claim 271, wherein the variant has a CTLA-4/CD28 binding affinity ratio greater than the CTLA-4/CD28 binding affinity ratio of human B7-1
273. The isolated or recombinant polypeptide variant of claim 271, wherein the variant has an ability to induce a T cell proliferation response that is about equal to or less than the T cell proliferation response induced by human B7-1.
274. An isolated or recombinant polypeptide variant comprising an amino acid sequence that differs from the amino acid sequence of a primate B7-2, wherein the

40032214-122001

difference between the amino acid sequence of the variant and the amino acid sequence of the primate B7-2 comprises a different amino acid at position 56 of the B7-2 polypeptide sequence shown at GenBank Acc. No. AAA58389 or at position 50 of the B7-2 polypeptide sequence shown at GenBank Acc. No. AAA86473.

5 275. The isolated or recombinant polypeptide variant of claim 274,
wherein the different amino acid is selected from the group of His, Arg, Lys, Pro, and/or
Trp.

276. The isolated or recombinant polypeptide variant of claim 275,
wherein the primate B7-2 is human B7-2 and the different amino acid is histidine.

10 277. The isolated or recombinant polypeptide variant of claim 276,
wherein the variant has a CTLA-4/CD28 binding affinity ratio greater than the CTLA-
4/CD28 binding affinity ratio of human B7-1 or B7-2.

15 278. The isolated or recombinant polypeptide variant of claim 276,
wherein the variant has an ability to induce a T cell proliferation
response that is about equal to or less than the T cell proliferation response induced by
human B7-1 or B7-2.

20 279. An isolated or recombinant polypeptide variant comprising an amino
acid sequence that differs from the extracellular domain (ECD) amino acid sequence of a
bovine B7-1, wherein the difference between the amino acid sequence of the variant and the
ECD amino acid sequence of the bovine B7-1 comprises a different amino acid at one or
more of the following amino acid residue positions: position 110, 124, 135, 192, 197, 199,
211, 213, 217, 218, 221, 225, 227, 231, 233, 235, 236, 237, 239, 240, 242, 243, and 244,
wherein each position corresponds to the position in the amino acid sequence of bovine B7-
1 of SEQ ID NO:280.

25 280. The isolated or recombinant polypeptide variant of claim 279,
wherein the difference between the amino acid sequence of the variant and the ECD amino
acid sequence of the bovine B7-1 comprises at least one of: (a) a different amino acid at
position 110, wherein the different amino acid is proline; (b) a different amino acid at
position 124, wherein the different amino acid is valine; (c) a different amino acid at
position 135, wherein the different amino acid is alanine; (d) a different amino acid at
position 192, wherein the different amino acid is valine; (e) a different amino acid at
position 197, wherein the different amino acid is glycine; (f) a different amino acid at
position 199, wherein the different amino acid is glutamic acid; (g) a different amino acid at

position 211, wherein the different amino acid is valine; (h) a different amino acid at position 213, wherein the different amino acid is asparagine; (i) a different amino acid at position 217, wherein the different amino acid is isoleucine; (j) a different amino acid at position 218, wherein the different amino acid is valine; (k) a different amino acid at 5 position 221, wherein the different amino acid is isoleucine; (l) a different amino acid at position 225, wherein the different amino acid is glutamic acid; (m) a different amino acid at position 227, wherein the different amino acid is serine; (n) a different amino acid at position 231, wherein the different amino acid is isoleucine; (o) a different amino acid at position 233, wherein the different amino acid is proline; (p) a different amino acid at 10 position 235, wherein the different amino acid is serine; (q) a different amino acid at position 236, wherein the different amino acid is lysine; (r) a different amino acid at position 237, wherein the different amino acid is proline; (s) a different amino acid at position 239, wherein the different amino acid is glutamine; (t) a different amino acid at position 240, wherein the different amino acid is glutamic acid; (u) a different amino acid at 15 position 242, wherein the different amino acid is proline; (v) a different amino acid at position 243, wherein the different amino acid is isoleucine; and (w) a different amino acid at position 244, wherein the different amino acid is aspartic acid.

281. The isolated or recombinant polypeptide variant of claim 280, 20 wherein the difference between the amino acid sequence of the variant and the ECD amino acid sequence of the bovine B7-1 further comprises at least one of: (1) a different amino acid at position 246, wherein the different amino acid is leucine; (2) a different amino acid at position 247, wherein the different amino acid is proline; (3) a different amino acid at position 248, wherein the different amino acid is phenylalanine; (4) a different amino acid at 25 position 250, wherein the different amino acid is valine; and (5) a different amino acid at position 253, wherein the different amino acid is proline, wherein each position corresponds to the position in the amino acid sequence of bovine B7-1 of SEQ ID NO:280.

282. The isolated or recombinant polypeptide variant of claim 280, further comprising a signal peptide.

30 283. The isolated or recombinant polypeptide variant of claim 282, further comprising at least one of a transmembrane domain signal peptide.

- 1003214-122002
284. A nucleic acid comprising a polynucleotide sequence that encodes an isolated or recombinant polypeptide of claim 269, 274, 279, 280, or 281, or a complementary nucleic acid sequence thereof.
285. An isolated or recombinant nucleic acid comprising a polynucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of the nucleic acid of claim 269, 274, 279, 280, or 281.
286. The isolated or recombinant polypeptide of claim 1 or 3, wherein the ECD amino acid sequence at least about amino acid residues 35 to 244 of any of SEQ ID NOS:48-68, 174-182, 184-221, 283-285, and 290-293.
287. The isolated or recombinant polypeptide of claim 1 or 3, which comprises a further amino acid sequence corresponding to at least one of a signal peptide, a transmembrane domain, or a cytoplasmic domain of a co-stimulatory polypeptide.
288. The isolated or recombinant polypeptide of claim 287, wherein the co-stimulatory polypeptide is a B7-1 polypeptide.
289. The isolated or recombinant polypeptide of claim 288, wherein the B7-1 polypeptide is a mammalian B7-1 polypeptide.
290. The isolated or recombinant polypeptide of claim 289, wherein the further amino acid sequence corresponding to the signal peptide sequence comprises at least about amino acid residues 1-34 of a polypeptide selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.
291. The isolated or recombinant polypeptide of claim 287, wherein the further amino acid sequence corresponding to the transmembrane domain comprises at least about amino acid residues 35-244, 35-243, or 35-242, 35-255, 35-254, or 35-253 of a polypeptide selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.
292. The isolated or recombinant polypeptide of claim 287, wherein the further amino acid sequence corresponding to the cytoplasmic domain comprises a cytoplasmic domain of a polypeptide selected from the group of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293.
293. The isolated or recombinant polypeptide of claim 1, which polypeptide comprises an ECD amino acid sequence encoded by an ECD coding nucleotide sequence, the ECD coding nucleotide sequence comprising a nucleotide sequence that, but

for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of the ECD coding nucleotide sequence of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142 or the nucleotide coding sequence that encodes the ECD of a polypeptide selected from any of SEQ ID NOS:48-68, 5 174-221, 283-285, and 290-293.

294. The isolated or recombinant polypeptide of claim 293, further comprising a signal peptide amino acid sequence encoded by a signal peptide coding nucleotide sequence, the signal peptide coding nucleotide sequence selected from the group of:

10 (a) a nucleotide sequence of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142, wherein said nucleotide sequence encodes a signal peptide;

(b) a nucleotide sequence that encodes the signal peptide of a polypeptide selected from any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and

15 (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

295. The isolated or recombinant polypeptide of claim 294, further comprising a transmembrane domain (TMD) amino acid sequence encoded by a TMD nucleotide sequence selected from the group of:

20 (a) a nucleotide sequence of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142, wherein said nucleotide sequence encodes a TMD polypeptide;

(b) a nucleotide sequence that encodes the TMD of a polypeptide selected from any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and

25 (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

296. The isolated or recombinant polypeptide of claim 295, further comprising a cytoplasmic domain (CD) amino acid sequence encoded by a CD nucleotide sequence selected from the group of:

30 (a) a nucleotide sequence of a polynucleotide sequence selected from any of SEQ ID NOS:1-21 and 95-142, wherein said nucleotide sequence encodes a CD polypeptide;

(b) a nucleotide sequence that encodes the CD of a polypeptide selected from any of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293; and

5 (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

297. The isolated or recombinant nucleic acid of claim 43, wherein the polynucleotide sequence of (d) comprises encodes a fragment of (a) or (b), wherein the fragment encodes an extracellular domain polypeptide having a CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of

10 human B7-1.

298. The isolated or recombinant polypeptide of claim 96, further comprising a signal peptide amino acid sequence encoded by a signal peptide coding nucleotide sequence, the signal peptide coding nucleotide sequence selected from the group of:

15 (a) a nucleotide sequence comprising a nucleotide fragment of a polynucleotide sequence selected from any of the group of SEQ ID NOS:22-45 and 143-173, wherein said nucleotide fragment encodes a signal peptide;

(b) a nucleotide sequence that encodes the signal peptide of a polypeptide selected from any of the group of SEQ ID NOS:69-92, 222-252, and 286-289; and

20 (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

299. The isolated or recombinant polypeptide of claim 298, further comprising a transmembrane domain (TMD) amino acid sequence encoded by a TMD nucleotide sequence selected from the group of:

(a) a nucleotide sequence of a polynucleotide sequence selected from any of the group of SEQ ID NOS:22-45 and 143-173, wherein said nucleotide sequence encodes a TMD polypeptide;

(b) a nucleotide sequence that encodes the TMD of a polypeptide selected from any of the group of SEQ ID NOS:69-92, 222-252, and 286-289; and

30 (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).

300. The isolated or recombinant polypeptide of claim 299, further comprising a cytoplasmic domain (CD) amino acid sequence encoded by a CD nucleotide sequence selected from the group of:
- (a) a nucleotide sequence of a polynucleotide sequence selected from any of the group of SEQ ID NOS:22-45 and 143-173, wherein said nucleotide sequence encodes a CD polypeptide;
- (b) a nucleotide sequence that encodes the CD of a polypeptide selected from any of the group of SEQ ID NOS:69-92, 222-252, and 286-289, and 290-293; and
- (c) a nucleotide sequence which, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of a nucleotide sequence (a) or (b).
301. An isolated or recombinant polypeptide comprising an amino acid sequence of at least an extracellular domain, wherein said extracellular domain amino acid sequence has at least about 75% amino acid sequence identity to an extracellular domain amino acid sequence of at least one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, and is not a naturally-occurring extracellular domain amino acid sequence, and wherein said polypeptide has an ability to induce T cell proliferation or T cell activation response that is equal to or greater than that of human B7-1.
302. The isolated or recombinant polypeptide of claim 1 or 301, wherein said polypeptide comprises more than one of the extracellular domain.
303. The isolated or recombinant polypeptide of claim 302, wherein said polypeptide comprises a multimer of the extracellular domain.
304. The isolated or recombinant polypeptide of claim 302, wherein said polypeptide comprises a fusion protein comprising at least one additional amino acid sequence.
305. The isolated or recombinant polypeptide of claim 304, wherein the at least one additional amino acid sequence comprises at least one Ig polypeptide.
306. An isolated or recombinant polypeptide comprising an amino acid sequence of an extracellular domain, wherein said extracellular domain (ECD) amino acid sequence has at least about 75% amino acid sequence identity to an extracellular domain amino acid sequence of at least one of SEQ ID NOS:48-68, 174-221, 283-285, and 290-293, and is not a naturally-occurring extracellular domain amino acid sequence, and wherein said polypeptide, in the presence of a population of activated T cells, has an ability to induce a T

cell proliferation or T cell activation response that is less than that induced by a human B7-1 ECD amino acid sequence in the presence of a population of activated T cells.

307. The isolated or recombinant polypeptide of claim 1 or 306, wherein said polypeptide comprises a soluble ECD monomer.

5 308. The isolated or recombinant polypeptide of claim 307, wherein said soluble ECD monomer further comprises an Ig polypeptide.

309. A soluble isolated or recombinant multimeric polypeptide comprising at least two polypeptides of claim 1 or 306.

10 310. The polypeptide of claim 309, wherein each of said at least two polypeptides further comprises at least one additional amino acid sequence which comprises an Ig polypeptide.

311. A nucleic acid comprising a polynucleotide sequence that encodes an isolated or recombinant polypeptide of claim 259, 269, 271, 275, 276, 279, 280, 301, or 15 306, or a complementary polynucleotide sequence thereof.

312. A vector comprising at least one nucleic acid which comprises a polynucleotide sequence that encodes an isolated or recombinant polypeptide of claim 259, 269, 271, 275, 276, 279, 280, 301, or 306, or a complementary polynucleotide sequence thereof.

20 313. A composition comprising a nucleic acid of claim 311 and a carrier.

314. A composition comprising a polypeptide of claim 259, 269, 271, 275, 276, 279, 280, 301, or 306 and a carrier.

25 315. A recombinant nucleic acid molecule comprising a polynucleotide sequence that, but for the degeneracy of the genetic code, hybridizes under at least stringent conditions over substantially the entire length of the nucleic acid of claim 311 or 312.

316. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide has an CD28/CTLA-4 binding affinity ratio about equal to or greater than the CD28/CTLA-4 binding affinity ratio of human B7-1.

317. The nucleic acid of claim 43, 44, or 46, wherein the polypeptide comprises a soluble polypeptide having an ability in the presence of a population of activated T cells to induce a T cell proliferation response that is less than the T cell proliferation response induced by a soluble human B7-1 polypeptide in the presence of a population of activated T cells.

318. The isolated or recombinant polypeptide of claim 171, wherein said polypeptide has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, and/or an ability to induce a T-cell proliferation or T-cell activation response about equal to or greater than that of hB7-1.

5 319. The isolated or recombinant nucleic acid of claim 170, wherein said nucleic acid encodes a polypeptide that has a CTLA-4/CD28 binding affinity ratio about equal to or greater than the CTLA-4/CD28 binding affinity ratio of human B7-1, and/or an ability to induce a T-cell proliferation or T-cell activation response about equal to or less than that of hB7-1.

10 320. The isolated or recombinant polypeptide of claim 1, 5, 6, or 7, wherein the polypeptide is bound to a cell membrane and has an ability to induce T-cell proliferation or T-cell activation or both.

Add B¹ >
Add B² >
Add B⁴ >